PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 116383	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU2003/001448**	International Filing Date *(day/month/year)* 3 November 2003	Priority Date *(day/month/year)* 4 November 2002
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C07K1/02, 1/14, 1/24, 1/26, 1/28, 1/30, 1/36		
Applicant PROTEOME SYSTEMS INTELLECTUAL PROPERTY PTY LTD et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand 14 May 2004	Date of completion of the report 17 February 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **D. A. LALLY** Telephone No. (02) 6283 2533

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001448

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	8, 11, 12, 18, 22 and 23	YES
	Claims	1 to 7, 9, 10, 13 to 17, 19 to 21, 24 to 26	NO
Inventive step (IS)	Claims	nil	YES
	Claims	1 to 26	NO
Industrial applicability (IA)	Claims	1 to 26	YES
	Claims	nil	NO

2. Citations and explanations (Rule 70.7)

Document 1: Mireles DeWitt C., Gomez G., and James J.M.. Journal of Food Science, (May 2002) 67 (9) 3335 to 3341.. "Protein extraction from beef heart using acid solubilization".

Document 2: Goto, M., *et al*: Biotechnology and Bioengineering, (1997) 54 (1) 26-32. "Design of surfactants suitable for protein extraction by reversed micelles."

Document 3: Macfarlane, D.E.: Anal Biochem, 1983 Jul 15; 132 (2): 231-5. "Use of benzyldimethyl-*n*-hexadecylammonium chloride ("16BAC"), a cationic detergent, in an acidic polyacrylamide gel electrophoresis system."

Document 4: Sato, T., *et al*: European Journal of Biochemistry: 1977 Sep; 78 (2): 557-67. "Membrane proteins of Escherichia coli K-12: two-dimensional polyacrylamide gel electrophoresis of inner and outer membranes."

Document 5: Mastro, R., *et al*: "Analytical Biochemistry (1999 Sep 10) 273 (2) 313-5. "Protein delipidation and precipitation by tri-butylphosphate, acetone, and methanol treatment for isoelectric focussing and two dimensional gel electrophoresis."

Document 6: Ono, T., *et al*: Biotechnology Progress, (1998 Nov-Dec) 14 (6) 903-8. "Factors affecting protein transfer into surfactant-isooctane solution: a case study of extraction behaviour of chemically modified cytochrome c."

Document 7: Gerstenfeld, L.C., *et al*: Calcified Tissue International, (1994 Sep) 55 (3) 230-5. "Selective extractability of noncollagenous proteins from chicken bone."

Document 8: Gilchrist, J.S., *et al*: Journal of Biological Chemistry (1993 Feb 25) 268 (6) 4291-9. "Identification and purification of a calcium binding protein in hepatic nuclear membranes."

Document 9: Nakaya, K., *et al*: Cancer Research, (1977 Oct) 37 (10) 3701-6. "Isolation and Some Biochemical Characteristics of Nuclei from AH-66 Hepatoma Cells."

Document 1:

This document describes the extraction of myofibrillar protein from beef heart. The process is principally a nonthermal acidic solubilization. In some instances, precipitation of the solubilized protein is prevented by the inclusion of 5% SDS. Citrate buffer is used. This recites all of the features of Claims 1 to 7, 9, 13 to17, 19 to 21, 24 to 26. I also consider the elements of the kit of claim 24 to be properly made out. Further to this, the choice of acidifying agent according to claims 8, 10, and 11 are arbitrary workshop equivalents and do not confer inventiveness over the art disclosed in this document. Also, the steps of degrading the compound and characterising it by routine physicochemical techniques are uninventive steps taken by the routineer in this field [Claims 22 and 23]. It should also be noted that the step of alkylating and reducing the peptide/protein is also a routine step in peptide isolation [see the alternatives in Claims 18 to 20]. As is the use of a chaotropic agent such as urea. In view of this document, Claims 1 to 7, 9, 13 to 17, 19 to 21, 24 to 26 are not novel, and Claims 1 to 26 lack an inventive step.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

- It is not readily apparent if Claims 17, and 19 to 21 are actually directed to processes for solubilising a proteinaceous macromolecule, or to a purification process to obtain a purified proteinaceous macromolecule.
- It is not readily apparent if Claim 22 is actually directed to processes for solubilising a proteinaceous macromolecule, or to a process for analysis or characterisation of this purified proteinaceous macromolecule.
- Claim 17 ought to read "The method according to any **one** of the...."

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

Document 2:

This document discloses the solvent extraction of proteins using a new class of surfactants that form reverse micelles. The document teaches that for protein extraction purposes that the surfactant itself has minimal solubility in water phase, and that the water content increases rapidly above pH 4, and that the pH of the reversed micellar phase is about 5.5. This recites all of the features of Claims 1, 2, 6, 7, 13 to 17, 19 to 21, 25 and 26. I also consider the elements of the kit of claim 24 to be properly made out. Further to this, the choice of acidifying agent according to claims 8 to 11 are arbitrary workshop equivalents and do not confer inventiveness over the art disclosed in this document. Also, the steps of degrading the compound and characterising it by routine physicochemical techniques are uninventive steps taken by the routineer in this field [Claims 22 and 23]. It should also be noted that the step of alkylating and reducing the peptide/protein is also a routine step in peptide isolation [see the alternatives in Claims 18, 19 and 20]. As is the use of a chaotropic agent such as urea. In view of this document, Claims 1, 2, 6, 7, 13 to 17, 19 to 21, 24 to 26 are not novel, and Claims 1, 2, 6 to 26 lack an inventive step.

Document 3:

This document teaches the use of benzyldimethyl-*n*-hexadecylammonium chloride as a solubilising agent for proteins, for subsequent gel electrophoresis. This solubilization is performed in acidic pH ranges, pH 4 is expressly mentioned. This recites all of the features of Claims 1 to 4, 6, 7, 13 to 17, 19 to 21, 25 and 26. I also consider the elements of the kit of claim 24 to be properly made out. Further to this, the choice of acidifying agent according to claims 8 to 11 are arbitrary workshop equivalents and do not confer inventiveness over the art disclosed in this document. Also, the steps of degrading the compound and characterising it by routine physicochemical techniques are uninventive steps taken by the routineer in this field [Claims 22 and 23]. It should also be noted that the step of alkylating and reducing the peptide/protein is also a routine step in peptide isolation [see the alternatives in Claims 18, 19 and 20]. As is the use of a chaotropic agent such as urea. In view of this document, Claims 1 to 4, 6, 7, 13 to 17, 19 to 21, and 24 to 26 are not novel, and Claims 1 to 4, and 6 to 26 lack an inventive step.

Document 4:

The membrane proteins of Escherichia coli K-12 were solubilized by the use of Triton X-100 with urea [a chaotropic agent] and ampholines [pH 3.5 to 10 and 5 to 7] and 2-mercaptoethanol. This recites all of the features of Claims 1 to 4, 6, 10, 12 to 17, 19 to 21, 25 and 26. I also consider the elements of the kit of claim 24 to be properly made out. Further to this, the choice of acidifying agent according to claims 7 to 11 are arbitrary workshop equivalents and do not confer inventiveness over the art disclosed in this document. Also, the steps of degrading the compound and characterising it by routine physicochemical techniques are uninventive steps taken by the routineer in this field [Claims 22 and 23]. It should also be noted that the step of alkylating and reducing the peptide/protein is also a routine step in peptide isolation [see the alternatives in Claims 18, 19 and 20]. As is the use of a chaotropic agent such as urea. In view of this document, Claims 1 to 4, 6, 10, 12 to 17, 19 to 21, 24 to 26 are not novel, and Claims 1 to 4, and 6 to 26 lack an inventive step.

Document 5:

This document discloses the solubilization of a protein [from the eggs of the tiger prawn *Penaeus monodon*] using surfactants, however, it is outside the range described/claimed in the present application. It does however show the use of urea as a chaotropic agent. In view of this I do not consider the invention of the present application to be either taught or suggested by this document and consider claims 1 to 26 to be both novel and inventive over this document.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

Document 6:

This document discloses the solubilization of a protein [cytochrome c] using surfactants, however, it is outside the range described/claimed in the present application. In view of this I do not consider the invention of the present application to be either taught or suggested by this document and consider claims 1 to 26 to be both novel and inventive over this document.

Document 7:

This document is silent upon the use of detergent/surfactant and because of this I do not consider it to be relevant. In view of this I do not consider the invention of the present application to be either taught or suggested by this document and consider claims 1 to 26 to be both novel and inventive over this document.

Document 8:

The Calcium-binding Protein [p93] was isolated using the surfactant /detergent Triton X-100. However, it appears to be attained at a pH range outside the scope of the claims of the present application. It does however show the use of urea as a chaotropic agent. In view of this I do not consider the invention of the present application to be either taught or suggested by this document and consider claims 1 to 26 to be both novel and inventive over this document.

Document 9:

Preparation/solubilization of the nuclei of AH-66 hepatoma ascite cells was attained by the use of non-ionic detergents including Triton X-100, Nonidet P-40 and Tween 80 as well as cetylpyridinium chloride. It would appear that they are buffered by Tris (pH 7.4) and thus outside the scope of the claims of the present application. In view of this I do not consider the invention of the present application to be either taught or suggested by this document and consider claims 1 to 26 to be both novel and inventive over this document.